regulation in the kidney, fluid circulation in ocular systems, gastric protection, water uptake by plants, etc. The field equations can be written with boundary conditions and parameters appropriate for the anatomy of each system. The field equations then form a physically and anatomically consistent model of biological function in the variational framework of modern field theory. The variational approach deals naturally with the many ionic solutions (containing a multitude of interacting components in a wide range of concentrations) and the wide range of conditions and forces used in experiments. Solving the PDEs will help suggest and interpret new experiments to understand the interaction of components, conditions, structure, and forces. In the view of classical physiology and biophysics, these interactions are the essence of biological function.

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Revisiting the Heteromultimeric Structure of ENaC in *XENOPUS Leavis* Oocytes

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The functional epithelial sodium channel (ENaC) is a heteromeric channel formed by three homologous alpha, beta and gamma subunits.

Several functional and biochemical studies have provided evidence that the ENaC is a heterotetramer formed by 2alpha, 1beta and 1gamma subunits. Recently, a high-resolution crystal structure has been obtained from an ortholog of ENaC, the acid-sensing ion channel ASIC1, which showed a homotrimeric channel. This discrepancy between two channels of the same ion channel family, motivated us to revisit the subunit oligomerization of ENaC.

His-tagged ENaC alpha, beta and gamma subunits were expressed in *Xenopus leavis* oocytes. The three ENaC subunits can be co-purified on Ni^{+2}-NTA agarose beads in an alpha beta gamma-ENaC complex. On Western blot, the ENaC subunits showed typical post-translation modifications associated with a functional channel. Using differentially tagged ENaC subunits, we investigated whether the ENaC complex contains more than a single alpha, beta or gamma subunits. Two differentially tagged alpha subunits co-purified with beta and gamma subunits, indicating that ENaC is formed by more than one alpha subunit. The purified ENaC complex channel eluted on Sephadex G 200 column in a fraction corresponding to a molecular weight of 350 kDa, which was higher than expected for a alpha beta gamma-ENaC.

These data confirm previous reports that the functional ENaC channel is a heterotetramer made of 2alpha 1beta, and 1gamma subunits.

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xshroom1 Regulates the Number of ENaC Channels Inserted in the Membrane of Oocytes From *XENOPUS Laevis*

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Shroom is a family of proteins linked to the actin cytoskeleton. We studied its effect upon the currents through ENaC channels (INa_{amil}) in oocytes (*X. laevis)* injected with α, β, and γ mENaC and xShroom1 sense or antisense oligonucleotides. We observed a strong reduction in INa_{amil} with the xShroom1 antisense: inward conductances (G_{inward}) (−160 to 0 mV) were 36 ± 12 μS and 1.80 ±.50 μS with xShroom1 sense and antisense. Similar results were obtained in oocytes expressing a mutant β-mENaC subunit (β-S518K) with a open probability of 1 (G_{inward} 65 ± 10 μS and 1.80 ± 2.0 μS for oocytes with xShroom1 sense or xShroom1 antisense. The negative effects of xShroom1 antisense can not be reversed with forskolin which reduced the rate of ENaC retrieval: G_{inward} : 124 ± 27 μS and 7.0 ± 1.9 μS with xShroom1 sense or xShroom1 antisense. Trypsin in the range of ng/ml activates the membrane-resident ENaC channels (Bengrine et al.2007), being this effect dependent on activation of G-proteins. Addition of 20 ng/ml of trypsin led to a slow increase in INa_{amil} with xShroom1 sense and it had no effect in most of the oocytes coinjected with ENaC and xShroom1 antisense (2 out of 20). Trypsin were without effects on the endogenous conductances. These data are consistent with the idea that the reduced INa_{amil} when xShroom1 is blocked is most probably due to a lack of functional ENaC channels in the plasma membrane.

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Synthetic Peptide-Based Channels: Candidates for Treatments of Channelopathies

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Modern approaches for treatments of channelopathies employ mutation-specific pharmacotherapies, use pharmacoagents alone, or a vast variety of ideas for genetic approaches, all with the purpose of restoring defective ion channels or attenuate their negative effects. An alternative to existing approaches is the use of synthetic channel-forming peptides (CFPs) with desirable selectivity, high ion transport rates and overall ability to supersede defective endogenous ion channels. Our synthetic CFPs represent derivatives of a peptide initially reconstituted from the second transmembrane segment of the α-subunit of Glycine receptor (M2GlyR), PARVGLGITTVLTMTTQSSGSRA. Due to poor efficiency of membrane insertion, tendency to form aggregates in aqueous solutions and inconsistent channel forming potentials, parent peptide sequences were modified to alter channel-inducing properties. Resulting NK_4-M2GlyR T19R, S22W, with two threonines replaced by a non-natural amino acid, diamino propionic acid (Dap) (NK_4-M2GlyR T19R, S22W, TT-Dap) represent our best candidates. We examined potential channel pore-lining residues using molecular dynamics studies followed by the sulfhydryl replacement technique. Identified residues involved in ion selectivity filter seem to represent a ring of β-hydroxyls from the threonines at position 17 and 13. Ussing chamber experiments employing MDCK cells and voltage-clamp studies using *Xenopus* oocytes revealed that introduction of Dap substitutions at pore-lining residues yields improved channel conductances. In conclusion, in addition to their ability to form soluble monomers in aqueous solutions, ability to self-assemble into homopentamers, efficient delivery to and insertion into the membranes of tested MDCK cells, NK_4-M2GlyR T19R, S22W, TT-Dap peptides show enhanced ion transport activities at low peptide concentration compared to their parent sequence (NK_4-M2GlyR T19R, S22W).

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Molecular Modeling and Simulation of a Synthetic Peptide Channel

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Many human diseases, including episodic ataxia, diabetes, epilepsy, cystic fibrosis and Alzheimer's dementia, are related to defective ion channels. A series of channel forming peptides derived from the second transmembran domain of the α1-subunit of the glycine receptor (M2GlyR) have been designed by the Tomich lab with improved anion conduction rate and aqueous solubility. To rationally understand the physiological properties of these synthetic channels and to identify improved designs, we combine NMR, biophysical data, and molecular modeling to provide a structural basis for understanding key physiochemical properties that govern the chloride conductivity and selectivity. Initial structural models were first constructed for one of our lead design, p22-T19R/S22W (KKKKP ARVGL GITTV LTMRT QW), primarily based on the monomer structure from solution NMR, amphipathicity consideration, and the oligomeric state of the channel assembly. Long molecular dynamic simulations in explicit membrane and water were then carried out to characterize the channel structural and dynamic properties. Interestingly, independent simulations from initial constructs with different handiness of helix packing (left, straight, and right) all converge to a similar structural ensemble with left-handed helix assembly. The predicted pore-lining residues are also in excellent agreement with a previous set of cysteine-scanning experiments. Coupled with parallel experimental characterizations in the Tomich lab, the simulation provides important insights into the structural basis of the activity of these synthetic channels.

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Two Genetic Variants of TRPM6 Increase Risk for Hypomagnesemia Associated with Diabetes Mellitus Type 2

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Diabetes mellitus type 2 (DM2) is characterized by high serum glucose levels, insulin resistance and hypomagnesemia. DM2-related hypomagnesemia has been linked to several chronic diabetic complications. Transient receptor potential melastatin 6 (TRPM6) channel plays an essential role in whole body Mg2+ homeostasis. It has been suggested by conducting nested case control study that two non-synonymous single nucleotide polymorphisms (SNP) of TRPM6, TRPM6(V1393I) & TRPM6(K1584E) might increase risk for DM2 in elderly women. Here, by measuring Total Glycosylated Hemoglobin (TGH) from 997 women in their last weeks of pregnancy as a measure of glucose control, we show that the two SNPs (6.8 %) have higher TGH compared to control subjects (6.3 %). Upon overexpression in a human kidney cell line, whole-cell patch clamp analysis showed that insulin activates (EC50 = 0.16 nM) TRPM6, but not the SNPs. Inability to phosphorylate T1391 was the identified factor for lack of insulin-mediated V1393I activation. We further demonstrate that TRPM6 stimulation is independent of its own kinase activity, but relied on both Phosphoinositide 3-kinase and Rac1. The impaired response of the SNPs to insulin may lead to hypomagnesemia causing insulin resistance. These SNPs could be used as markers to improve diagnosis and identify those at risk for developing DM2.